PATENT
Attorney Docket No. EXT-026 (2457/23)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S):	Shuber		
SERIAL NO.:	09/545,162	GROUP NO.:	1634
FILED:	April 7, 2000	EXAMINER:	J. Switzer
TITLE	METHODS FOR DETECTING NUCLEIC ACIDS INDICATIVE OF CANCER		

DECLARATION OF ANTHONY P. SHUBER UNDER 37 C.F.R. § 1.132

I, Anthony P. Shuber, hereby declare as follows:

1. I am Executive Vice President and Chief Technology Officer at Exact Sciences Corporation, and I am an inventor named on the above-captioned patent application, U.S.S.N. 09/514,162 (hereafter "the Application").

2. I have extensive experience in the area of molecular biology, and especially molecular diagnostics, as evidenced by the copy of my curriculum vitae attached as Exhibit A.

3. I have read and understood the Application, including currently-pending claims 7-14.

4. I make this declaration in order to provide my scientific opinions and facts known to me which may be of assistance in the examination of the Application.

5. My colleagues and I have conducted several clinical trials to assess parameters of screening assays described and claimed in the Application. I present below summaries of the methodology we used, and the results we obtained using methods described and claimed in the Application.

6. Essentially, I received from the Mayo Clinic (Rochester, Minnesota) stool samples obtained from Mayo Clinic patients. I screened those samples using methods disclosed and claimed in the Application, specifically methods directed toward screening for the presence of at least a nucleic acid characteristic of cancer or precancer, such as those described in claims 7, 8, and 9. According to those methods, patients who have disease (in this case cancer) produce bodily fluid or excretions that contain large (relative to healthy patients) amounts of high-integrity (intact or relatively intact) nucleic acids.

7. My colleagues and I at Exact Laboratories attempted to amplify human genomic DNA in each stool sample we received using PCR primer pairs spaced 200bp, 400bp, 800bp, 1.3kb, 1.8kb, or 2.4kb apart, essentially as described in Examples 1 and 2 of the Application. According to this screening assay, an amount of amplifiable DNA that is statistically greater than the amount expected in a sample obtained from a healthy patient was a positive screen.

8. We determined the amount of DNA amplified using each set of primers, and then decoded the diagnostic information regarding each patient from whom a sample was obtained. We did this to determine whether the results of our screening assay predicted which patients had disease.

9. In one study, the assay, directed towards screening for the presence of at least a nucleic acid characteristic of cancer or precancer, successfully screened for 16 out of 27 patients who had colon cancer and 18 out of 27 patients who had cancer outside the colon and who were colonoscopically negative. Exhibit B provides results for patients suspected of having colon cancer. Exhibit C provides a list of the non-colonic cancers for which screening was successful in this study and a summary of the data. These include cancer of the pancreas, lung, bile duct, stomach, duodenum, and esophagus.

10. In another study, 15 out of 25 patients who had cancers outside the colon (and who were colonoscopically negative) were screened as positive using the methods directed towards screening for the presence of at least a nucleic acid characteristic of cancer or precancer in stool samples. Exhibit D provides a summary of those data.

11. In a third study, methods directed towards screening for the presence of at least a nucleic acid characteristic of cancer or precancer were used to screen additional patient stool samples. Those methods accurately screened 14 out of 25 non-colonic cancers. A summary of those data are provided in Exhibit E.

12. In each of the studies described above, methods directed towards screening for the presence of at least a nucleic acid characteristic of cancer or precancer accurately diagnosed negative stool samples from healthy patients between 72% and 100% of the time.

13. Based upon my data described above, I believe that nucleic acid integrity is a marker for cancer or precancer generally. Overall, the sensitivity of the assay directed towards screening for the presence of at least a nucleic acid characteristic of cancer or precancer is 78.4%. This is a much higher sensitivity than is typically observed in other screening assays, such as fecal occult blood testing, PSA testing, and mammography.

14. Based upon my experience in the field of molecular diagnostics and my above-described data, I believe a positive screen for cancer or precancer can be established when a nucleic acid fragment in a sample containing exfoliated cells is of a length that is greater than a length expected in a sample from a healthy patient. The reason for my belief is that patients who have disease (in this case, cancer or precancer) produce bodily fluid or excretions that contain, in relation to healthy patients, large amounts of intact or relatively intact nucleic acids.

15. Based upon my experience in the field of molecular diagnostics and my above-described data, I believe that the assays described and claimed in the Application are capable of screening for any type of cancer or precancer.

16. Based upon my experience in the field of molecular diagnostics and my above-described data, I believe that the assays described and claimed in the Application can be conducted in

any bodily excretion, body fluid sample, or tissue. The reason for my belief is that samples such as stool, pus, sputum, blood and the like all contain the cellular debris that is measured by my assays. In a patient having a disease, I believe that any bodily excretion, tissue, or body fluid sample contains high-integrity nucleic acid that can be measured according to the claims of the Application.

17. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the Application or any patent issued thereon.

Dated: November 10, 2003

Anthony P. Shuber

Exhibit A

Anthony P. Shuber

11 Grant Street
Milford, MA 01757
(508) 478-5492

PROFESSIONAL EXPERIENCE

EXACT LABS — 6/96 - Present

Vice President, Molecular Biology (1/98 - Present)
Director, Molecular Biology (6/96 - 1/98)
Supervisor: Stanley N. Lapidus, President

- Responsible for the development of DNA based molecular technologies utilized in a stool based screening test for the early detection of sporadic colorectal cancer.

GENZYME GENETICS / INTEGRATED GENETICS, FRAMINGHAM, MA — 4/90 - 6/96

Senior Scientist, Manager, Technical Development Laboratory (10/93 - 6/96)
Supervisor: Dr. Katherine Klinger, Vice President of Science

- Responsible for the identification, development, and validation of new tests and technologies to be applied in various areas of clinical diagnostics including cytogenetics, molecular cytogenetics, biochemistry, and DNA diagnostics. This includes managing two doctoral level research scientists, eleven research associates, their performance evaluations, and their career development.

Research Scientist II, Manager, Technical Development Laboratory (4/92 - 10/93)
Supervisor: Dr. Katherine Klinger, Vice President of Science

- Established and managed a new technical development laboratory made up of six research associates. This included performance evaluations and career development planning for all six research associates.
- Developed a quantitative PCR procedure as an analytical method for determining purity and yield of fetal cells isolated from maternal blood following various methods of separation.
- Developed, validated and introduced a DNA based diagnostic test for Gauchers disease.
- Expanded a previously developed method of identifying point mutations within the CFTR gene from 12 to 32 mutations.

Supervisor, DNA Diagnostic Laboratory (4/90 - 4/92)
Supervisor: Dr. Barbara Handelin, Director

- Managed multiple areas of laboratory operations involved with the DNA diagnosis of 10 genetic diseases. This included the management of five technologists, their performance reviews, and their career development.
- Managed daily operations, trouble shooting technical difficulties, and the QC/QA involved with the clinical area of molecular diagnostics.
- Designed and developed a novel detection system involved in determining the molecular weights of VNTR fragment length polymorphisms.
- Developed a more cost effective and informative test for identifying point mutations within the CFTR (cystic fibrosis transmembrane conductance regulator) gene.

DAMON BIOTECH, Needham Heights, MA — 8/89 - 4/90

Research Scientist, Molecular Biology Department
Supervisor: Dr. Kin Ming Lo, Director

- Constructed recombinant chimeric monoclonal antibodies and subsequent expression in myeloma cells for the use in human therapeutics.

EG&G BIOMOLECULAR, Natick, MA 2/89 - 6/89

Scientist, Molecular Biology Department
Supervisor: Dr. Frances Toneguzzo

- Investigated different sequencing techniques in order to apply double strand sequencing and PCR sequencing to an automated DNA sequencer.

GENETICS INSTITUTE, Cambridge, MA 9/83 - 2/89

Scientist, Molecular Biology Department (9/87 - 2/89)
Supervisor: Dr. Dave Rogers, Director

- Developed a hybridization procedure that can be utilized in cloning novel gene family related sequences relying solely on nucleic acid sequence homology, and to better understand the properties and limitations of different hybridization conditions where perfect sequence homology does not exist. As a model for our study, coding sequence for a fibroblast growth factor related gene was used.
- Optimized and performed a tissue culture assay in order to better understand the biochemistry of this particular growth factor as well as any novel factor identified using this hybridization method.

Scientist, Molecular Biology Department (9/86 - 9/87)
Supervisor: Dr. John Wozney, Senior Scientist

- Cloned, characterized and analyzed human genomic sequences within a bone growth factor gene family utilizing synthetic oligonucleotides complementary to regions of high homology within the bone growth factor family.

Associate Scientist 8, Bacterial Expression Department (11/84 - 9/86)
Promoted from Assistant Scientist to Associate Scientist 2 to Associate Scientist 8
Supervisor: Dr. Paul Schendel, Director

- Cloned, characterized, and expressed prokaryotic genes. Designed cloning strategies, characterized the genomic organization and determined the gene sequence coding for E. coli products aspC, aspA, ilvE, and dnak, as well as the gene sequences coding for the M. formicicum gene product formate dehydrogenase. The genes were expressed in E. coli , the expression optimized, and the recombinant protein products analyzed.

Research Associate, Bacterial Expression Department (9/83 - 11/84)
Supervisor: Dr. John McCoy, Research Scientist

- Constructed plasmid expression vectors and analyzed recombinant expression products, including the subcloning, expression and characterization of human TPA synthesized in E. coli.

AWARDS/HONORS

Recipient of Outstanding Leadership Award (Genzyme / Integrated Genetics)	3/96
Recipient of President's Eagle Award (Genzyme / Integrated Genetics)	11/93
Recipient of scholarship/teaching assistantship	9/80 - 7/83

EDUCATION

M.S., Biology, Marquette University, Milwaukee, Wisconsin July 1983

Master's Research Project: Identification and Analysis of cDNA clones Complementary to Abundant Oocyte-Specific Transcripts in Drosophila melanogaster.

B.S., Biology, Marquette University, Milwaukee, Wisconsin May 1980

PUBLICATIONS

Diana W. Bianchi, John M. Williams, Lisa M. Sullivan, Frederick W. Hanson, Katherine W. Klinger, **Anthony P. Shuber.** (1997) PCR Quantitation of Fetal Cells in Maternal Blood in Normal and Aneuploid Pregnancies. Am. J. Hum. Genet. 61: 822-829

Anthony P. Shuber, Lesley A. Michalowsky, G. Scott Nass, Joel Skoletsky, Lisa M. Hire, Steve K. Kotsopoulos, Dana M. Barberio, Michael F. Phipps, Katherine W. Klinger. (1996). Complex Mutation Analysis Using a Multiplex Allele-Specific Diagnostic Assay (MASDA). In Preparation.

Diana W. Bianchi, Katherine W. Klinger, Theresa J. Vadnais, Mary Ann DeMaria, **Anthony P. Shuber,** Joel Skoletsky, Pat Midura, Matthew DiRiso, Christine Pelletier, Michelle Genova, Marlena Erikson and John M. Williams. (1996). Development of a Model System to Compare Cell Separation Methods for the Isolation of Fetal Cells from Maternal Blood. Prenatal Diagnosis. 16: 289-298

Steve K. Kotsopoulos and **Anthony P. Shuber.** (1996) Isolation of 3.5-kb Fragments on Magnetic Solid Supports. BioTechniques 20: 198-200

Anthony P. Shuber, Valerie J. Grondin, and Katherine W. Klinger, (1995). A Simplified Procedure for Developing Multiplex PCRs. Genome Research. 5 (5): 488-493

Barbara Stewart, Joseph Zabner, **Anthony P. Shuber,** Micheal J. Welsh, and Paul B. McCray, Jr (1995). Normal Sweat Chloride Values Do Not Exclude the Diagnosis of Cystic Fibrosis. Am J. Respir. Crit. Med. 151: 899-903

Diana W. Bianchi, MD, **Anthony P. Shuber, MS,** Mary Ann DeMaria, BS, Arthur C. Fougner, MD, and Katherine W. Klinger, Ph.D. (1994). Fetal cells in maternal blood: Determination of purity and yield by quantitative polymerase chain reaction. Am. J. Obstet. Gynecol. 171(4): 922-926

Kiesewetter, S., Macek Jr, M., Davis, C., Curristin, S.M., Chu, C-S., Graham, C., Shrimpton, A.E., Cashman, S.M., Tsui, L-C., Mickle, J., Amos, J., Highsmith, W.E., **Shuber, A.** , Witt, D.R., Crystal, R.G., Cutting, G.R. (1993). A mutation in CFTR produces different phenotypes depending on chromosome background. Nat. Gen. 5(3): 274-279

Shuber, A.P. , Skoletsky, J., Stern, R., Handelin, B.L. (1993). Efficient 12-mutation testing in the CFTR gene: a general model for complex mutation analysis. Hum Mol Genet. 2(2):153-158.

Richards, B., Skoletsky, J., **Shuber, A.P.** , Balfour, R., Stern, R.C., Dorkin, H.L., Parad, R.B., Witt, D., Klinger, K.W. (1993). Multiplex PCR amplification from the CFTR gene using DNA prepared from buccal brushes/swabs. Hum. Mol. Genet. 2(2):159-163.

Shuber, A.P. , Orr, E.C., Recney, M.A., Schendel, P.F., May, H.D., Schauer, N.L., Ferry, J.G., (1986). Cloning, expression, and nucleotide sequence of the formate dehydrogenase genes from Methanobacterium formicicum. J. Biol. Chem. 261(28):12942-12947.

ABSTRACTS/CONFERENCE PAPERS

B. Allitto, P. Finn, N. Napolitano, M. Sysma, E. Sugarman, J. Ekstein, G. Miller, **A. Shuber**. Prevalence of the 1604A Gaucher Disease Mutation in the General Ashkenazi Jewish Population. (1997) 46th Annual Meeting, ASHG

B. Allitto, R. Heim, J. DeMarchi, L. Michalowsky, **A. Shuber**, M. Girardi. (1996) High Through-put Clinical Testing for 70 CF Mutations. 45th Annual Meeting, ASHG

Diana W. Bianchi, **Anthony P. Shuber**, Christine Pelletier, Katherine W. Klinger, John W. Williams. (1996). Fetal Cell Quantitation In Maternal Blood Samples from Normal and Aneuploid Pregnancies. Annual Meeting of the Society for Pediatric Research.

Burschyk, Monica ; **Shuber, T.** ; Skoletski, J. ; Neusser, M. ; Garritsen, H.S.P. ; Holzgreve, W. Ganshirt, D. (1995) PCR Detection of fetal Cells in Maternal Blood. 27th Annual Meeting of the European Society of Human Genetics (ESHG)

V. Grondin, K. Klinger, **T. Shuber**. (1994) A Simple Procedure Eliminating Multiple Optimization Steps Required in Developing Multiplex PCR Reactions. 43rd Annual Meeting, ASHG

D.W. Bianchi, **A.P. Shuber**, M.A. DeMaria, K. W. Klinger. (1994) Use of dual PCR to quantify fetal cells isolated from maternal blood. 43rd Annual Meeting, ASHG

Bianchi, D.W., **Shuber, A.P.** , DeMaria, M., Fougner, A.C., Klinger, K.W. (1994). Fetal Cells in Maternal Blood: Determination of Purity and Yield by Quantitative PCR. 14th Annual Society for Perinatal Obstetrics.

Williams, J.M., Erikson, M.S., Pelletier, C., Weil, G.J., **Shuber, T.** , Mahr, A., Klinger, K.W., Bianchi, D.W. (1992). Comparison of cell separation methods to enrich the proportion of fetal cells in maternal blood samples. 42nd Annual Meeting, ASHG

Allitto, B., **Shuber, A.** , Sugarman, E., DiMaggio, R., Handelin, B. (1992). Prenatal diagnosis of CF in fetuses with ultrasound indications of abnormal bowel. 42nd Annual Meeting, ASHG

Handelin, B.L., Witt, D., Skoletsky, J., **Shuber, A.** (1992). Unexpected prevalence of R117H and G551D CF mutations in a randomly screened population. 42nd Annual Meeting, ASHG

Sugarman,E., **Shuber,A.** , DiMaggio, R., Munsat, T.L., Brustowicz, L.M., Gilliam, T.C., Handelin, B. (1992). Prenatal diagnosis of Spinal Muscular Atrophy experience in first 56 families. 42nd Annual Meeting, ASHG

Fry-Mehltretter, L., Skoletsky, J., **Shuber, T.** , Handelin, B. (1991) DNA banking experience for multiple disease indications at Integrated Genetics. 8th International Congress, ASHG

Book Chapters

Handelin, Barbara and **Shuber, Anthony P.** (1994). Simultaneous Detection of Multiple Point Mutations Using Allele-Specific Oligonucleotides. Current Protocols In Human Genetics. Ann L. Boyle, Ph.D., Ed. John Wiley & Sons, Inc.

Patents

Direct Sequence Identification of Mutations by Cleavage- and Ligation-Associated Mutation-Specific Sequencing
Patent #: 5,707,806
Issued: 1/13/98
Inventors: **Shuber, Anthony P.**

Method for the Detection of Clonal Populations of Transformed Cells in a Genomically Heterogeneous Cellular Sample
Patent #: 5,670,325
Issued: 9/23/97
Inventors: Lapidus, Stanley N., **Shuber, Anthony P.**, Ulmer, Kevin M.

Method for Simultaneously Detecting Multiple Mutations in a DNA Sample
Patent#: 5,633,134
Issued: 5/27/97
Inventors: **Shuber, Anthony P.**

High-Throughput Screening Method for Sequence or Genetic Alterations in Nucleic Acids Using Elution and Sequencing of Complementary Oligonucleotides
Patent#: 5,589,330
Issued: 12/31/96
Inventors: **Shuber, Anthony P.**

Method for Mismatch-Directed In Vitro DNA Sequencing.
Patent#: 5,571,676
Issued: 11/5/96
Inventors: **Shuber, Anthony P.**

Other Patents Pending

EXHIBIT B

Patient No.	Score	Screen Result
P-064	30	+
P-103	30	+
P-104	30	+
P-108	30	+
P-101	29	+
P-102	29	+
P-099	28	+
P-107	28	+
P-110	26	+
P-098	25	+
P-134	24	+
P-062	23	+
P-090	23	+
P-095	23	+
P-093	22	+
P-100	21	+
P-122	18	-
P-084	15	-
P-109	15	-
P-118	10	-
P-138	10	-
P-091	8	-
P-096	8	-
P-053	7	-
P-119	6	-
P-117	5	-
P-105	0	-

EXHIBIT C

Patient No.	Type of Cancer	Age	Score	Screen Result
P-145	Pancreas	68	30	+
P-164	Lung CA	68	30	+
P-166	Bile Duct	52	30	+
P-189	Bile Duct	43	30	+
P-190	Lung CA	50	30	+
P-019	Atypical Findings in Stomach	71	29	+
P-152	Lung CA	77	28	+
P-167	Pancreas	72	28	+
P-011	Lung CA	73	27	+
P-153	Pancreas	65	27	+
P-165	Lung CA	85	27	+
P-170	Duodenum	65	27	+
P-182	Barrett's Esophagus	58	27	+
P-146	Bile Duct	63	26	+
P-081	Barrett's Esophagus	74	26	+
P-151	Pancreas	49	25	+
P-155	Lung CA	60	25	+
P-156	Lung CA	57	25	+
P-150	Pancreas	78	23	+
P-149	Esophagus	59	19	-
P-154	Esophagus	80	19	-
P-169	Pancreas	71	19	-
P-168	Lung CA	63	18	-
P-180	Pancreas	67	13	-
P-144	Esophagus	59	9	-
P-147	Stomach	57	7	-
P-148	Stomach	69	6	-

EXHIBIT D

Patient No.	Type of Cancer	Score	Screen Result
P-166	Bile Duct	24	+
P-139	Bile Duct	24	+
P-146	Bile Duct	22	+
P-170	Duodenum	23	+
P-154	Esophagus	17	-
P-149	Esophagus	14	-
P-144	Esophagus	8	-
P-171	Esophagus	0	-
P-147	Gastric	8	-
P-148	Gastric	7	-
P-152	Lung	24	+
P-164	Lung	24	+
P-165	Lung	24	+
P-190	Lung	24	+
P-011	Lung	22	+
P-155	Lung	19	-
P-156	Lung	18	-
P-168	Lung	18	-
P-145	Pancreas	24	+
P-151	Pancreas	21	+
P-153	Pancreas	21	+
P-167	Pancreas	21	+
P-150	Pancreas	20	+
P-169	Pancreas	16	-
P-160	Pancreas	6	-

EXHIBIT E

Patient No.	Type of Cancer	Score	Screen Result
V-48	Lung, Squamous	24	+
V-37	Pancreas Adenocarcinoma	23	+
V-41	Bile Duct Adenocarcinoma	23	+
V-46	Gallbladder Adenocarcinoma	23	+
V-42	Esophageal Adenocarcinoma	21	+
V-59	Lung, Squamous	21	+
V-38	Pancreas Adenocarcinoma	20	+
V-52	Lung, Squamous	17	+
V-63	Lung Adenocarcinoma	16	+
V-44	Cholangiocarcinoma	13	+
V-62	Pancreas Adenocarcinoma	13	+
V-47	Esophagus, Squamous	10	+
V-50	Lung Adenocarcinoma	9	+
V-67	Pancreas Adenocarcinoma	9	+
V-51	Lung Adenocarcinoma	3	-
V-65	Lung Adenocarcinoma	4	-
V-04	Lung Adenocarcinoma	5	-
V-40	Esophageal Adenocarcinoma	4	-
V-61	Lung Adenocarcinoma	4	-
V-57	Lung Adenocarcinoma	4	-
V-26	Stomach Adenocarcinoma	3	-
V-54	Stomach Adenocarcinoma	3	-
V-55	Lung Adenocarcinoma	3	-
V-58	Lung, Squamous	4	-
V-66	Lung Adenocarcinoma	2	-